



Subtle shifts in microbial communities occur alongside the release of carbon induced by drought and rewetting in contrasting peatland ecosystems

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1 Subtle shifts in microbial communities occur
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34 **Abstract**

35 Peat represents a globally significant pool of sequestered carbon. However, peatland carbon
36 stocks are highly threatened by anthropogenic climate change, including drought, which leads
37 to a large release of carbon dioxide. Although the enzymatic mechanisms underlying
38 drought-driven carbon release are well documented, the effect of drought on peatland
39 microbial communities has been little studied. Here, we carried out a replicated and
40 controlled drought manipulation using intact peat ‘mesocosm cores’ taken from bog and fen
41 habitats, and used a combination of community fingerprinting and sequencing of marker
42 genes to identify community changes associated with drought. Community composition
43 varied with habitat and depth. Moreover, community differences between mesocosm cores
44 were stronger than the effect of the drought treatment, emphasising the importance of
45 replication in microbial marker gene studies. While the effect of drought on the overall
46 composition of prokaryotic and eukaryotic communities was weak, a subset of the microbial
47 community did change in relative abundance, especially in the fen habitat at 5 cm depth.
48 ‘Drought-responsive’ OTUs were disproportionately drawn from the phyla *Bacteroidetes* and
49 *Proteobacteria*. Collectively, the data provide insights into the microbial community changes
50 occurring alongside drought-driven carbon release from peatlands, and suggest a number of
51 novel avenues for future research.

52 **Introduction**

53 Anthropogenic climate change is one of the key issues of the 21st century, with the potential
54 to severely impact human lives as well as natural ecosystems¹. The effect of climate change
55 on soil biodiversity and consequent ecological processes is of particular concern because of
56 the potential for detrimental positive feedback effects. An overarching concern is that
57 warming can lead to an increase in soil respiration and consequently an increased release of
58 carbon dioxide into the atmosphere²⁻⁵. Micro-organisms should be considered when

59 attempting to understand and predict the effects of climate change on soil processes, since
60 microbial communities are central to the decomposition of soil organic matter⁶ and are
61 directly responsible for a large proportion of soil respiration⁷. In addition, microbial
62 communities play a key role in determining gas fluxes⁸ and rates of nutrient cycling⁹. While it
63 is difficult to separate effects which are mediated by changes to soil microbial communities
64 from the direct effects of environmental change, there is strong evidence that the soil
65 microbial community is important in determining the way that soil processes respond to
66 environmental change¹⁰⁻¹².

67 Peat soils are an extremely important global store of carbon: estimates for the total amount of
68 carbon stored in Northern peatlands vary from 273 Gt C to 547 Gt C^{13,14}. However, climate
69 change represents a serious threat to temperate peatlands; for example, within the UK the
70 area covered by blanket peat is projected to decline, with the potential for peatlands to change
71 from carbon sinks to carbon sources¹⁵. Likewise, the amount of carbon stored within
72 peatlands in Canada¹⁶, the USA¹⁷ and across the Northern hemisphere¹⁸ is also predicted to
73 decline.

74 The effect of drought on carbon cycling within peatlands has been an area of particular
75 interest. While climate change models project an increase in total precipitation at high
76 latitudes, rainfall is likely to become more concentrated in extreme events interspersed with
77 periods of dry weather¹⁹, while higher temperatures will increase water loss from soils²⁰.
78 Together, these effects will lead to an increase in the likelihood of drought events^{19,20} and a
79 fall in peatland summer water table^{21,22}. Unlike drier habitats, where drought leads to
80 moisture-limiting conditions and a reduction in carbon release by heterotrophic micro-
81 organisms⁶, drought in peatlands facilitates the aeration of previously anaerobic peat layers.
82 Aeration stimulates microbial decomposition, and consequently leads to increased carbon
83 dioxide release²³. The effect of drought on peatland carbon dioxide fluxes often outlasts the

84 duration of the drought itself by a considerable margin due to the degradation of inhibitory
85 phenolic compounds under anaerobic conditions²³. Therefore, the effects of increased
86 summer drought frequency on peatland carbon fluxes represent a potential positive feedback
87 loop, with the potential to accelerate rates of global warming. There is some evidence that the
88 composition of peatland microbial communities responds to long-term water table changes²⁴⁻
89 ²⁶, with Actinobacteria and fungi responding particularly strongly after several years of water
90 table drawdown²⁴. Microbial community composition also changes in response to short-term
91 drought²⁷⁻²⁹, although the exact microbial groups involved remain unclear. Moreover, while
92 protozoa have been neglected in modern-day studies of drought effects on peatland microbial
93 communities, paleoecological studies indicate that testate amoebae community composition
94 in peat is strongly influenced by water table depth³⁰. Nevertheless, the microbial mechanisms
95 underlying drought-driven carbon dioxide release from peat remain poorly understood.

96 The development of high-throughput sequencing-based approaches for the identification of
97 microorganisms has provided an unprecedented opportunity to advance our understanding of
98 microbial communities in natural environments, and to explore the effects of environmental
99 change on these communities. Initial DNA-based microbial ecology studies were limited by
100 the low throughput of existing sequencing methodologies or the low resolution of
101 ‘community fingerprinting’ approaches³¹, but the introduction of high-throughput sequencing
102 platforms immediately decreased the cost per base pair of sequencing data. Lower sequencing
103 costs and paradigm shifts in throughput have enabled sequencing of rRNA genes to be used
104 on much broader scales and across a wide spectrum of biological diversity³².

105 In order to identify changes in microbial communities which occur concurrently to the release
106 of carbon from peat ecosystems, here we aimed to use high-throughput marker gene
107 sequencing to identify the proportion of the microbial biosphere which is affected by drought
108 and rewetting in bogs and fens, two habitats which are representative of the majority of

109 temperate peatlands in the Northern hemisphere. A replicated and controlled drought
110 manipulation was carried out using peat ‘mesocosm cores’ collected from both habitats (Fig.
111 1). In addition to the concurrent monitoring of greenhouse gas fluxes, DNA was extracted
112 and purified from two contrasting depths below the peat surface. Extracted DNA was then
113 subjected to automated ribosomal intergenic spacer analysis (ARISA), a community
114 fingerprinting technique enabling rapid and low-cost estimation of diversity within a
115 microbial community, in order to confirm that drought affected microbial communities.
116 ARISA fingerprinting was followed by sequencing, bioinformatics and statistical analysis of
117 16S and 18S rRNA genes to obtain a more detailed perspective of community changes.

118 **Results**

119 ARISA fingerprinting of bacterial communities yielded bands ranging in size from 110-2839
120 bp, while ARISA fingerprinting of fungal communities yielded bands ranging from 54-2851
121 bp. Binning of ARISA amplicons into 5 bp bins gave a total of 185 bins for bacterial
122 communities and 87 for fungal communities.

123 Sequencing yielded a total of 102,439,895 and 104,156,662 paired-end reads for 16S and 18S
124 rRNA genes, respectively. Of the 16S rRNA gene reads, 29,337,117 passed quality control
125 steps and were clustered into 49,892 OTUs. Of the 18S rRNA gene reads, a total of
126 17,214,346 passed quality control and paired-end joining, which were clustered into 43,058
127 OTUs. Following standardisation of read numbers, rarefaction curves were generated to
128 assess sequencing coverage (Fig. S1) and these suggested that sequencing coverage was
129 adequate, particularly for samples from the bog.

130 **Effects of Habitat and Depth on Microbial Community**

131 NMDS ordination of ARISA fingerprinting data showed some separation of samples taken
132 from bog mesocosm cores at 20 cm depth from other habitats and depths (Fig. 2a). Fungal
133 communities were more weakly affected by habitat and depth, but samples taken from the

134 bog at 5 cm appeared to be distinct from all other samples on the third axis (Fig. 2b).
135 PERMANOVA tests confirmed that ARISA fingerprinting profiles of both bacterial and
136 fungal communities were significantly affected by habitat (bacteria: $P=0.001$; fungi:
137 $P=0.001$; Table S1) and depth (bacteria: $P=0.001$; fungi: $P=0.001$; Table S1) although in
138 each case the effect size (R^2 value) was small (Table S1), indicating that habitat and depth
139 only accounted for a small proportion of overall variation. Bacterial communities were also
140 significantly affected by the interaction between habitat and depth ($P=0.001$; Table S1).

141 Sequencing of 16S and 18S rRNA genes identified an effect of habitat and depth on both
142 prokaryotic (16S) and eukaryotic (18S) communities that was stronger than that detected by
143 ARISA fingerprinting. For both markers, samples clustered by habitat along the first axis and
144 by depth along the second axis (Fig. 2c; 2d). PERMANOVA tests confirmed that there were
145 significant effects of habitat (16S: $P=0.001$; 18S: $P=0.001$; Table S2), depth (16S: $P=0.001$;
146 18S: $P=0.001$; Table S2) and the interaction term (16S: $P=0.001$; 18S: $P=0.001$; Table S2) on
147 community composition.

148 Seven prokaryotic phyla and six eukaryotic phyla each made up $>1\%$ of reads in at least one
149 habitat and depth (Fig. 3a; 3b). *Acidobacteria* and *Proteobacteria* contributed by far the
150 highest proportions of prokaryotic reads in both the bog and the fen: *Acidobacteria*
151 contributed 47% of reads in the bog but only 13% in the fen, while *Proteobacteria* contributed
152 20% of reads in the bog and 19% in the fen. However, a large proportion of prokaryotic
153 OTUs could not be assigned to phylum level at the requisite *utax* confidence level of 0.85,
154 with fen communities containing a higher proportion of ‘Unassigned’ OTUs than bog
155 communities.

156 Within the eukaryotic communities, an even higher proportion of the community could not be
157 assigned. In particular, at 20 cm depth 90% of reads belonged to OTUS which could not be

158 assigned to phylum level at the chosen confidence level (0.85). Amongst OTUs which could
159 be assigned, the highest numbers of reads were contributed by Chloroplastida (green plants;
160 9% of reads in the bog and 3% in the fen) and Fungi (11% of reads in the bog and 6% in the
161 fen).

162 Linear mixed effect models were fitted to transformed proportional abundances of reads from
163 the most abundant phyla in order to determine which factors affected phylum-level
164 community composition. Of the seven prokaryotic phyla which made up >1% of the
165 community, all but *Verrucomicrobia* were significantly affected by habitat and depth, and all
166 were significantly affected by the interaction between habitat and depth (Table S3). In
167 particular, *Acidobacteria* made up a higher proportion of reads in the bog and at 5 cm depth;
168 *Proteobacteria* made up the highest proportion of reads in the bog at 5 cm and the lowest in
169 the bog at 20 cm; and *Bacteroidetes* made up the highest proportion of reads in the fen and at
170 5 cm depth (Fig. 3a). Conversely, three of the six eukaryotic phyla tested were significantly
171 affected by habitat (Alveolata, Stramenopiles, Rhizaria), four were affected by depth (Fungi,
172 Alveolata, Metazoa, Rhizaria), and four were affected by the interaction between habitat and
173 depth (Fungi, Alveolata, Stramenopiles, Rhizaria; Table S4). Reads assigned to phyla
174 Alveolata, Rhizaria and Stramenopiles were all more abundant in the fen than the bog. Reads
175 assigned to Fungi, Alveolata and Rhizaria were each more abundant at 5 cm than 20 cm.

176 Effect of Drought and Environmental Variables on Microbial Communities

177 Under drought conditions and during rewetting, treated mesocosm cores had significantly
178 higher redox potentials and significantly lower water content than control mesocosm cores
179 (Fig. 4d; Fig. S2; Table S5). Carbon dioxide fluxes rose significantly during drought but
180 returned to control levels during rewetting, while methane fluxes fell and remained
181 suppressed throughout the rewetting period (Fig. 4a; 4b; Table S6). The concentration of
182 dissolved organic carbon (DOC) was significantly lower in fen mesocosm cores than in bog

183 mesocosm cores, and was lower in droughted mesocosm cores (pre-drought measurements of
184 DOC concentration were not taken; Fig. 4c; Table S6). However, there was also an
185 unexpected rise in the water content of the peat between the first two sampling time points
186 (Fig. S2). There was a significant effect of treatment on bacterial ARISA fingerprinting
187 profiles in the bog at both depths and in the fen at 20 cm (Table S7), while the effect of
188 treatment on the fungal community was only significant in the fen at 5 cm. There was a
189 significant two-way interaction between time point and treatment on fungal communities in
190 the fen at 20 cm. In addition, prokaryotic communities at 20 cm in both habitats changed
191 significantly between sampling time points (Table S7) and on fungal communities in the bog
192 at both depths and in the fen at 20 cm (Table S7). However, sequencing of 16S and 18S
193 rRNA genes indicated that there was no effect of the drought-rewetting treatment on overall
194 community composition. NMDS ordinations of these communities indicated that the
195 mesocosm core from which samples were taken had a stronger effect on community
196 composition than time point or treatment (Fig. 5). PERMANOVA tests confirmed this
197 observation: while community composition was significantly different between treatments,
198 neither time point nor the interaction effect had a significant effect (Table S8) and therefore
199 the treatment effect observed in sequencing data was likely due to pre-existing differences
200 between the mesocosm cores assigned to each treatment (Fig. 5). Conversely, the effect of
201 core was strongly significant in all habitats and depths and for both markers (Table S9).
202 Application of envfit confirmed differences in microbial communities between mesocosm
203 cores, and also found significant correlations between vegetation and the prokaryotic
204 community (Table 1; Fig. 5). Prokaryotic community composition was significantly
205 correlated to CO₂ fluxes in the bog at 5 cm depth and the fen at 20 cm depth (although
206 significance was marginal in the latter case), while methane fluxes were not significantly
207 correlated to community composition (Table 1). Fewer significant correlations existed

208 between environmental variables and the community composition of microbial eukaryotes,
209 although there was a weak correlation between eukaryotic community composition and the
210 concentration of phenolic compounds in both habitats at 20 cm depth (Table 1).

211 None of the seven most abundant prokaryotic phyla showed significant changes in relative
212 abundance in response to drought (Table S3). Of the six most eukaryotic phyla, only the
213 relative abundance of Rhizaria was significantly affected by drought, showing an increase in
214 abundance when the water table reached its minimum in the fen at 5 cm depth before falling
215 again during rewetting (interaction between time point and treatment: $F_{8,177}=2.6$, $P=0.009$;
216 Fig. 6; Table S4).

217 Following abundance filtering of all OTUs, linear mixed effect models were fitted in order to
218 detect OTUs which were significantly affected by the interaction between time point and
219 treatment (hereafter ‘drought-affected OTUs’). Drought-affected OTUs are summarised in
220 Table 2, and full details given in Tables S10-S13 and Figs. S3-S6. Briefly, far more drought-
221 affected OTUs were detected in the fen at 5 cm than in any other habitat and depth; in the fen
222 at 5 cm, 37 prokaryotic OTUs and 7 eukaryotic OTUs showed significant changes in relative
223 abundance during drought. Conversely, the number of drought-affected prokaryotic OTUs in
224 other habitats and depths ranged from 2-5 OTUs, while the number of drought affected
225 eukaryotic OTUs ranged from 1-3. NMDS ordination of only drought-affected OTUs
226 confirmed that the effect of drought was most consistent in the fen at 5 cm (Fig. S7).

227 Amongst drought-affected OTUs in the fen at 5 cm, the phyla *Proteobacteria* and
228 *Bacteroidetes* were overrepresented relative to their abundance in the dataset as a whole:
229 *Proteobacteria* made up 27% of the overall community and 41% of drought-affected OTUs,
230 while *Bacteroidetes* made up only 7% of the overall community but 39% of drought-affected
231 OTUs. The majority of the drought-affected OTUs which were assigned to *Bacteroidetes*

232 showed a negative response to drought while the majority of those assigned to *Proteobacteria*
233 responded positively, but there were exceptions to this pattern. Few OTUs could be assigned
234 to genus level, but negatively drought-affected OTUs included likely members of genera
235 *Paludibacter* and *Geobacter* while positively drought-affected OTUs included members of
236 genera *Massalia*, *Duganella* and *Caulobacter*. Eukaryotic drought-affected OTUs in the fen
237 at 5 cm contained members of the Alveolata, Rhizaria and Nematoda, as well as four OTUs
238 which could not be assigned at phylum level (Table 2).

239 Very few drought-affected OTUs occurred in the other habitats. From the 16S rRNA gene
240 dataset, there were five drought-affected OTUs in the fen at 20 cm depth, four in the bog at 5
241 cm and two in the bog at 20 cm depth. Amongst these, *Acidobacteria* and Unassigned
242 *Bacteria* were the most common taxonomic assignments (Table 2).

243 **Discussion**

244 While differences in microbial community composition between habitats and depths were
245 detected in analyses based on both ARISA fingerprinting and amplicon sequencing data, the
246 effect of habitat and depth was much stronger when community analysis was based on
247 sequencing data (Fig. 2). The greater resolution in SSU rRNA sequencing data likely results
248 from the limitations of ARISA fingerprinting, which is based on intraspecies differences in
249 the length of the intergenic spacer region of ribosomal rRNA genes. However, in highly
250 diverse environments such as soils, multiple species can share the same intergenic spacer
251 length³¹, reducing the resolution of this technique.

252 The phylum-level composition of microbial communities in both habitats was similar to
253 previous studies of peat soils³³⁻³⁵, suggesting that the composition of peatland communities is
254 conserved across geographically disparate regions, at least at the level of phylum. Relative
255 abundances of all abundant bacterial phyla were significantly affected by both habitat and
256 depth, while only a subset of eukaryotic phyla exhibited demonstrable differences in

257 community composition between habitats and depths. However, phyla containing microbial
258 eukaryotes (Fungi, Stramenopiles, Rhizaria and Alveolata) were more strongly affected by
259 habitat and depth than were macrofaunal phyla, likely because the methods used were not
260 designed to detect variations in the abundance of macrofaunal organisms. Additionally, the
261 large proportion of eukaryotic reads belonging to OTUs which could not be annotated to
262 phylum level likely made differences in abundance more difficult to detect. The strong effect
263 of habitat on the relative abundance of many phyla is unsurprising given that almost all
264 measured environmental variables differed between the two habitats; in comparison to the fen
265 mesocosm cores, bog cores had lower mean pH values and redox potentials, but much higher
266 concentrations of DOC.

267 Within each habitat and depth, there were significant differences in the community
268 composition of the mesocosm cores, potentially linked to differences in environmental
269 variables between different cores. In particular, the percentage cover of different plant
270 functional groups was significantly correlated to microbial community composition in several
271 cases, as were the concentration of phenolic compounds and the pH of the peat. Plants are an
272 important driver of microbial communities and are able to influence the rhizosphere
273 microbiome directly, for example *via* root exudates³⁶. Alternatively, plant communities can
274 act as more effective indicators of soil chemistry over longer time periods, compared to
275 insights derived from a single snapshot in time of microbial community composition³⁷.

276 As expected, the drought treatment led to a rise in redox potential and a corresponding release
277 of carbon dioxide while both methane flux and the concentration of DOC fell, corresponding
278 to the results of previous studies^{21,23}. However, unlike previous studies, carbon dioxide fluxes
279 in in droughted mesocosm cores immediately returned to similar levels as observed in control
280 cores when rewetting began, despite the fact that the redox potential remained elevated. The
281 fall in carbon dioxide flux as the water table rises may result from carbon dioxide dissolving

282 in the porewater rather than being released at the surface of the peat; the concentration of
283 dissolved inorganic carbon (DIC) increases rapidly on rewetting³⁸, suggesting the potential
284 for porewater to absorb the gases released by microbial metabolism. Alternatively, carbon
285 dioxide release due to increased respiration by autotrophs during drought cannot be ruled out;
286 in some cases, root respiration increases following aeration of peat³⁸. Unexpectedly, the water
287 content of the peat rose between the first and second time points in all habitats and at all
288 depths (Fig. S2). The reasons for this rise are unclear as the mesocosm cores were transferred
289 to bins of water within hours of collection, with small holes drilled for water exchange.
290 However, the mesocosm cores in the current experiment were larger than those used in
291 previous studies³⁹, creating a potential mechanism for less efficient water exchange between
292 cores and the surrounding water.

293 Despite the clear effect of drought and rewetting on carbon cycling, the effect of the drought-
294 rewet treatment on microbial community composition was weak and overshadowed by
295 differences between mesocosm cores. ARISA fingerprinting suggested a significant, albeit
296 weak, effect of drought within certain depths and habitats, but there was no corresponding
297 effect in the sequence-based analysis. This discrepancy may have arisen as a result of
298 differences in the lengths of amplicons measured by each method: ARISA amplicons were
299 165-1,580bp long, while sequenced rRNA amplicons were 300-350bp. In freshwater lakes,
300 seasonal changes in community composition derived from analysis of invertebrate
301 environmental DNA have been more rapidly detected when analysing smaller amplicons⁴⁰, as
302 the size distribution of DNA becomes more skewed towards smaller fragments over time⁴¹.

303 The weak response of microbial communities to drought and rewetting in both datasets
304 suggests that the increased carbon dioxide flux observed during drought was not mediated by
305 changes in microbial community composition. In addition, CO₂ fluxes were only significantly
306 correlated to prokaryotic community composition in two of the four possible combinations of

307 habitat and depth (Table 1), and in both cases the correlation was weak (Fig. 5). However, it
308 is possible that members of the microbial community changed in activity rather than
309 abundance, or that genuine community changes were obscured by DNA belonging to
310 dormant or dead organisms^{42,43}. Although not feasible in the present study,
311 metatranscriptomic analyses would further clarify the relative contributions of shifts in the
312 active versus the overall community to drought⁴⁴. While metatranscriptomic analysis has yet
313 to be applied to temperate peatlands, in permafrost peatlands metatranscriptomic analysis
314 gives subtly different results to metagenomics, and so it is likely that differences exist
315 between the active community and the DNA present in soil⁴⁵. In addition, awareness of the
316 role played by rare species in community function and response to environmental change has
317 recently begun to increase^{46,47}; due to the difficulties in separating genuinely rare OTUs from
318 erroneous reads, rare OTUs were not the focus of this study, but it is possible that future
319 studies could gain new insights by focusing on the rare portion of the microbial biosphere in
320 peat ecosystems.

321 While amplicon sequencing suggested that drought and rewetting did not affect overall
322 community composition, there were nonetheless indications that individual groups of micro-
323 organisms responded to the treatment. In particular, phylum Rhizaria (a phylum of protists)
324 made up a significantly higher proportion of the community in the fen at 5 cm at minimum
325 water table (Fig. 6). The response of Rhizaria to drought is of potential interest, as protists
326 may play important roles in mediating the response of environmental processes to
327 environmental change. For example, grazing by ciliates may determine the rate of change in
328 bacterial biomass under warming conditions⁴⁸ while a fall in the abundance of mixotrophic
329 testate amoeba led to a rise in peatland carbon dioxide emissions following warming⁴⁹.
330 Rhizaria also play an important role in the export of carbon from marine planktonic

331 systems⁵⁰. The role played by protists (especially Rhizaria) in the context of bottom up and
332 top down controls in the carbon cycle of droughted peatlands therefore merits further study.

333 Testing for significant effects of the drought-rewet treatment on individual prokaryotic OTUs
334 revealed that the relative abundance of a number of OTUs changed relative to control
335 conditions during drought and/or rewetting, particularly in the fen at 5 cm depth. A large
336 proportion of ‘drought-affected OTUs’ in the fen at 5 cm depth belonged to *Bacteroidetes* and
337 *Proteobacteria*. Notably, both of these phyla have been previously identified as containing a
338 high proportion of non-dormant cells when compared to other bacterial phyla⁴³, potentially
339 meaning that they more rapidly respond to environmental change by increasing or decreasing
340 in abundance rather than activity. Only two negatively drought-affected OTUs could be
341 assigned to genus level: one of these belonged to genus *Paludibacter*, the sole described
342 member of which is an obligately anaerobic fermenter⁵¹, and the other to *Geobacter*, a genus
343 of metal-reducers. Therefore, a number of obligate anaerobes may fall in abundance in the
344 active layer of fens following drought. Patterns were more difficult to detect amongst the
345 positively drought-affected OTUs, many of which belonged to the *Proteobacteria*, a diverse
346 phylum containing a broad range of functional categories⁵². Intriguingly, two positively
347 drought-affected OTUs were affiliated with taxa that are commonly associated with
348 petroleum-contaminated soils: genus *Caulobacter* and family *Sphingomonadaceae*^{53,54}. Both
349 taxa contain aerobic bacteria, prompting speculation that aeration during drought may allow
350 proliferation of bacteria involved in aerobic degradation of organic matter. However, it should
351 be noted that few were significant following the application of corrections for multiple
352 comparisons and thus this analysis should be viewed as a hypothesis-generating rather than a
353 confirmatory study.

354 Collectively, the current study highlights an array of important insights into the microbial
355 mechanisms underpinning the drought-driven release of carbon from globally important peat

356 ecosystems. The replicated design and enhanced taxonomic resolution afforded by the marker
357 gene analyses demonstrated marked heterogeneity between putatively similar experimental
358 cores. Furthermore, the study suggests that drought-driven changes in carbon fluxes in
359 peatland ecosystems are not associated with large-scale community changes, and thus raises
360 the possibility that these changes may be caused by shifts in the activity rather than the
361 composition of the microbial community or may be a result of small shifts in beta diversity
362 which have large effects on community function. We predict that future combinations of
363 metagenomic and metatranscriptomic analyses will yield further insights to complement
364 existing theories and highlight biogeochemical mechanisms that could be targeted to enhance
365 carbon retention in globally important peat ecosystems.

366 **Materials and Methods**

367 **Collection of Mesocosm Cores and Experimental Design**

368 Mesocosm cores were collected from two sites representing typical temperate bog and fen
369 habitats. Fen cores were extracted from Cors Erddreiniog, a low-lying fen in mid-Anglesey,
370 North Wales, UK (grid reference SH461826), which is designated a Special Area of
371 Conservation and represents a nationally important area of alkaline and calcareous fen habitat
372 (JNCC 2007). Bog cores were taken from Marchlyn Mawr (NVC classification M6 [*Carex*
373 *echinata* – *Sphagnum recurvum/auriculatum* mire]⁵⁵), on the outskirts of Snowdonia National
374 park (grid reference SH610625). Marchlyn Mawr was chosen because of its proximity to
375 important drinking water reservoirs.

376 Peat ‘mesocosm cores’ were collected in lengths of PVC pipe (each 20 cm in diameter and 35
377 cm in length), following a protocol adapted from that of Freeman, Lock and Reynolds³⁹.
378 After collection, mesocosm cores were kept in a controlled temperature room at 8-10 °C for
379 the duration of the experiment and lit by fluorescent daylight tubes (mean PAR: 305.4 μmol
380 $\text{m}^{-2} \text{sec}^{-1}$) on a 16:8 hour day-night cycle. Cores were placed in bins which were filled to the

381 level of the peat surface with artificial rainwater for bog cores and artificial groundwater for
382 fen cores, with holes drilled near the base of each core to allow water exchange with the
383 surrounding water. The composition of the rainwater followed a standard recipe⁵⁶, while the
384 groundwater was produced following a custom recipe that emulated the chemical
385 composition of groundwater at Cors Erddreiniog according to earlier measurements (Table
386 S14).

387 Within each habitat, five of the ten mesocosm cores were randomly assigned to the drought-
388 rewet treatment while the remaining five acted as controls. The water table in the control
389 cores was level with the surface of the peat throughout, mimicking field conditions, while the
390 water table in drought cores at each sampling time point is described in Table S15 and in Fig.
391 1. Briefly, the first two time points were simulated as ‘pre-drought’, during which the water
392 table in each mesocosm core was level with the peat surface. Following the pre-drought, the
393 water table in treatment cores was gradually lowered over nine weeks, kept stable at 20 cm
394 below the peat surface for six weeks, and rewetted over six weeks (Table S15). The length
395 and intensity of the drought treatment was based on a natural drought which occurred in 2006
396 in the Cerrig-yr-Wyn catchment in mid-Wales²³. Additionally, all mesocosm cores were
397 allowed to acclimatise in the controlled temperature room for approximately one month prior
398 to the first sampling time point.

399 Sample Collection and DNA Extraction

400 Soil and gas samples were collected at three week intervals (Table S15). Gas samples were
401 taken between 10am and noon following methods previously used to analyse carbon fluxes
402 from peat mesocosm cores²³. Briefly, a sealed headspace was placed over each mesocosm
403 core. A 20 cm³ gas sample was removed from the headspace at 0, 15, 30, 60 and 120 minutes
404 and injected into an evacuated 12 ml glass vial (Labco Medical Supplies). Gas samples were
405 analysed on a Varian 450-GC fitted with a flame ionisation detector (FID) and a methaniser.

406 At each time point, the machine was calibrated using three gas mixtures of known
407 concentration obtained from Scientific and Technical Gases Ltd (Newcastle under Lyme,
408 Staffordshire, UK). In each case, a linear regression line was calculated between time and
409 carbon dioxide concentration and the slope of the regression was taken as the average flux
410 value.

411 Subsequently, six gram samples of peat were collected at 5 cm (chosen to correspond to the
412 most biogeochemically active layer of the peat) and 20 cm (chosen to correspond to
413 minimum water table) below the peat surface. Immediately after the removal of soil samples,
414 the redox potential of the peat was measured using a redox probe with an Ag/AgCl reference
415 electrode in 3 M KCl. To adjust the value obtained to the 'true' value (i.e. that which would
416 have been obtained using a standard hydrogen electrode), a correction factor of +207 was
417 added prior to further analysis⁵⁷. The hole from which peat samples were taken were
418 immediately plugged to prevent water loss and destruction of the peat structure. The size of
419 the cores was sufficient that each sampling event removed only a small proportion of the total
420 material, and no subsistence of mesocosm cores was observed over the course of the
421 experiment.

422 The soil samples were homogenised thoroughly using flame-sterilised tools before DNA was
423 extracted from a 0.25 g subsample using a MoBio PowerSoil kit (Cambio, Cambridge),
424 following manufacturer's instructions. Following preliminary tests, DNA extracted with the
425 MoBio PowerSoil kit was found to contain lower levels of PCR inhibitors than alternative
426 methods. DNA was eluted into 100 μ L sterile Tris-EDTA buffer (10 mM Tris, 1 mM EDTA,
427 pH 7.6) and stored at -80 °C prior to further analysis. Samples were further purified using a
428 MoBio PowerClean kit following manufacturer's instructions, as purification was found to
429 result in more consistent PCR amplification during downstream molecular biological
430 manipulation.

431 Percentage water content of peat was measured by weighing a subsample of peat before and
432 after drying at 108 °C for 48 hours. Phenol oxidase activity was assayed using the phenolic
433 amino acid L-3,4- dihydroxy phenylalanine (L-DOPA) as a substrate, as described in detail
434 by Dunn *et al.*⁵⁸. The concentration of phenolic compounds was assayed using Folin-
435 Ciocalteu Reagent⁵⁹: briefly, a 1 cm³ subsample of peat was taken using a cut-off syringe and
436 weighed. Water-soluble phenolics were extracted by homogenising the peat subsample with 9
437 ml of water before centrifuging the resulting slurry. 250 µl of supernatant was added to each
438 of three wells of a clear microplate and baseline absorbance measured prior to addition of
439 12.5 µl Folin-Ciocalteu reagent and 37.5 µl filtered sodium carbonate solution (200 mg l⁻¹).
440 Samples were mixed, incubated at room temperature for 90 minutes, and absorbance
441 measured at 750 nm. A calibration curve was produced using dilutions of phenol solution in
442 the range of 0-10 mg l⁻¹. A pH meter was inserted into peat slurry (1 g peat: 9 ml water) in
443 order to measure the pH of the peat. DOC measurement was carried out using a Thermalox
444 TOC/TN analyser equipped with a non-dispersive infrared CO₂ detector.

445 ARISA Fingerprinting

446 Automated ribosomal intergenic spacer analysis (ARISA) is a community fingerprinting
447 technique enabling rapid and low-cost estimation of diversity within a microbial community.
448 The method involves amplifying the intergenic spacer region of microbial ribosomal DNA
449 and analysing the length of the obtained amplicons. The length of the intergenic spacer region
450 is very variable, and amplicons of different sizes are therefore expected to represent separate
451 species or strains³¹. Although all cores were used in biogeochemical analyses (N=5), this was
452 not possible for nucleic acid-based analyses, and so within each combination of time point
453 and treatment a subset of three of these mesocosm cores were selected for ARISA
454 fingerprinting and all downstream molecular genetic work. Within each chosen core, ARISA

455 fingerprinting was carried out on samples taken from both depths and all nine time points,
456 giving a total of 216 samples for this part of the analysis.

457 The primers employed for ARISA of bacterial communities were ITSF (5'-
458 GTCGTAACAAGGTAGCCGTA-3') and ITSReub (5'-GCCAAGGCATCCACC-3'), which
459 have been shown to outperform other commonly used ARISA primers⁶⁰. As there was no
460 existing comparison of primer pairs for ARISA of fungal communities, selected primers were
461 tested using Primer Prospector software⁶¹. Based on this comparison, a combination of
462 ITS1WH (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-
463 TCCTCCGCTTATTGATATGC-3') was selected.

464 Each ARISA PCR reaction contained 9.45 µl of nuclease-free water, 12.5 µl of PCR Master
465 Mix (Promega), 1 µl of each primer (10 µM), 0.05 µl molecular grade bovine serum albumin
466 (1mg/ml, Thermo Scientific) and 1 µl template DNA (diluted to 10 ng/µl) to give a final
467 volume of 25 µl. For ARISA fingerprinting of bacterial communities, thermal cycling
468 parameters were 95 °C for 2 minutes for initial denaturation, followed by thirty cycles of 95
469 °C for one minute (denaturation), 52 °C for 45 seconds (annealing), 72 °C for 1.5 minutes
470 (extension), and a final extension period of five minutes. An annealing temperature of 54.2
471 °C was used for the fungal ARISA primers, with all other steps in the PCR program identical
472 to those for the bacterial communities. PCR amplicon lengths were measured on a Qiaxcel
473 Advanced (Qiagen), using a Qiaxcel High Resolution kit and method OM1200
474 (recommended by the manufacturer for amplicon lengths between 0.5 and 1.5 kbp).

475 Sequencing of 16S and 18S rRNA Genes

476 As with ARISA fingerprinting, sequencing of rRNA genes could only be carried out for
477 samples taken from a subset of mesocosm cores: for consistency, the same three mesocosm
478 cores were chosen for sequencing as for ARISA fingerprinting. Library preparation,

479 sequencing of 16S and 18S rRNA genes and initial quality control was carried out by the
480 Earth Microbiome Project⁶² (<http://www.earthmicrobiome.org/>) according to standard
481 protocols. Briefly, the V4 region of the 16S rRNA gene was amplified using primers 515f
482 (5'-GTGCCAGCMGCCGCGGTAA-3') and 806r (5'-GGACTACHVGGGTWTCTAAT-
483 3')⁶³, which amplify both bacterial and archaeal sequences, and the V9 region of the 18S
484 rDNA gene was amplified using Illumina_Euk_1391f (5'-GTACACACCGCCCGTC-3') and
485 Illumina_EukBr (5'-TGATCCTTCTGCAGGTTACCTAC-3')⁶⁴. Sequencing was carried
486 out on an Illumina HiSeq in rapid run mode, giving paired-end reads of 150bp in length.
487 Quality control and demultiplexing was carried out in QIITA (<http://qiita.microbio.me/>), a
488 QIIME-based repository and analysis platform for “-omics” data, and was equivalent to
489 quality control in QIIME using default parameters.

490 Sequence Processing and operational taxonomic unit (OTU) Clustering

491 Following removal of poor quality reads by the Earth Microbiome Project, further quality
492 control and OTU clustering was carried out in VSEARCH⁶⁵, a method which has been
493 proven to output high quality OTUs⁶⁶, followed by taxonomic assignment using USEARCH
494 v8.12⁶⁷. VSEARCH was run on the HPC Wales system. Identical reads were merged and *de*
495 *novo* chimera prediction was carried out using UCHIME, as implemented in VSEARCH,
496 with default parameters. Next, chimeras were manually removed and OTUs were clustered
497 within VSEARCH at 97% similarity, and an OTU table suitable for downstream analysis was
498 generated using the script ‘uc2otutab.py’ (http://drive5.com/python/uc2otutab_py.html).
499 Taxonomy was assigned to each OTU using the ‘utax’ command in USEARCH v8.12⁶⁷.
500 Taxonomy was assigned against the provided UTX reference data for 16S rRNA genes,
501 which is based on RDP training set v15⁶⁸, and against the SILVA database v111 for 18S
502 rRNA genes⁶⁹.

503 Where large differences in read numbers exist between samples and differences between
504 treatments are subtle, rarefaction has been shown to perform outperform other methods of
505 normalization prior to clustering analyses (e.g. NMDS ordination)⁷¹. Thus, prior to further
506 analysis, read numbers were standardised in all samples using the ‘rrarefy’ command from
507 the ‘VEGAN’ package⁷⁰. Samples in the 16S rRNA gene dataset were standardised to contain
508 70,000 reads each and samples in the 18S rRNA gene dataset were standardised to contain
509 20,000 reads each. The thresholds used for standardisation were chosen to include the
510 majority of samples, but exclude samples where sequencing had failed. Samples which
511 contained fewer reads than these thresholds were removed from the dataset: 10 samples were
512 removed from the 16S rRNA gene dataset and 9 from the 18S rRNA gene dataset as they did
513 not contain the requisite number of reads for the read number standardisation step.

514 Statistical Analyses

515 The experimental design gave rise to four independent variables: habitat, depth, treatment and
516 time point. To test for significant effects of these variables on fluxes of carbon dioxide (CO₂)
517 and methane (CH₄) and on the concentration of dissolved organic carbon (DOC), linear
518 mixed effect models were fitted using package ‘nlme’ in R⁷². Linear mixed effect models are
519 widely applicable in ecological analyses⁷³, and were required in this case to allow for the
520 effect of mesocosm core. Since multiple samples were taken from each mesocosm core,
521 analyses would otherwise have been confounded by temporal pseudoreplication. Model
522 selection was based on the recommendations of Zuur *et al.*⁷³. Briefly, models were initially
523 fitted with all main effects (habitat, depth, treatment, and time point) and all two and three-
524 way interactions included. Mesocosm core was included as a random effect. Interaction
525 effects were removed sequentially based on hypothesis testing using a likelihood ratio test
526 until only significant interactions remained (with the exception of the interaction between

527 time point and treatment, which was kept in all models due to the importance of this term to
528 the focal aims of the study).

529 Community data from ARISA fingerprinting was analysed using the ‘vegan’ package in R⁷⁰.
530 First, fragment sizes were sorted into 5bp bins and converted to presence-absence data. Next,
531 NMDS ordination was carried out on Jaccard distances across all samples (appropriate for
532 binary (presence-absence) data) followed by PERMANOVA tests.

533 Following standardisation of sequence numbers across samples, OTU abundance tables were
534 subject to the same multivariate analyses as ARISA fingerprinting data, but based on Bray-
535 Curtis dissimilarity rather than binary Jaccard distances, due to the semi-quantitative
536 information included in this kind of data⁷⁴. In order to focus on the community composition
537 of microbial eukaryotes, all OTUs in the 18S rRNA dataset which were assigned to phyla
538 Holozoa, Chloroplastida and Metazoa (at any confidence level) were excluded from
539 calculations of NMDS ordinations and PERMANOVA tests. Unassigned OTUs were also
540 removed. Results of NMDS ordination were linked to environmental variables using function
541 ‘envfit’ from package ‘VEGAN’⁷⁰: this was done within habitat-depth subsets due to the
542 strong effect of habitat and depth on both microbial community composition and
543 environmental variables. Envfit first calculates the direction of the effect of a given variable:
544 for ‘vectors’ (continuous variables) this is done by calculating the direction of maximum
545 correlation between the variable and the ordination scores, while for ‘factors’ (discrete
546 variables) envfit calculates the average ordination score for each factor level. Next,
547 significance values are calculated for each variable using a permutation test.

548 To test for significant effects of habitat, depth, treatment and time point on the most abundant
549 phyla (i.e. those which made up >1% of the community), the proportion of each phylum was
550 logit-transformed⁷⁵ and linear mixed effect models were fitted as described above for gas
551 fluxes.

552 Linear mixed effect models were also used to identify individual OTUs that showed
553 significant responses to drought. Within each habitat-depth combination, OTUs were first
554 filtered to include only those OTUs which were sufficiently abundant (at least 1 read per
555 1000 reads in one sample) and present in at least 20% of samples. This strict filtering was
556 carried out in order to minimise effects of rare OTUs; the high proportion of rare OTUs in the
557 dataset was considered likely to generate spurious results. Following filtering, the relative
558 abundance of each OTU was logit transformed⁷⁵ and linear mixed effect models were fitted
559 with mesocosm core as a random effect (random intercept model). Benjamini-Hochberg
560 corrections⁷⁶ were calculated to correct for the large number of comparisons. Where a
561 significant effect of the interaction between time point and treatment was found, OTU
562 abundances were carefully scrutinised and cases where the interaction effect was due to
563 outlier effects were removed.

564

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756

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762 **Author Contributions**

763 All authors reviewed the manuscript. CP performed the experiment and analysed the data.
764 SC, CF, NF, JM and PG conceived of the experiment and contributed to the discussion of
765 results. TJ, LM & AE contributed DOC data and analysis. GA carried out the sequencing and
766 initial quality control of sequencing data. CP wrote the manuscript, and all authors
767 contributed to review of the manuscript.

768 **Competing Financial Interests**

769 We are not aware of any competing financial interests.

770 **Data Accessibility**

771 Sequence data is publically archived on the ENA/EBI database (accession
772 number ERP016584) and on QIITA (<https://qiita.ucsd.edu/>; study ID:10278).

773

774 **Table 1** Results of ‘envfit’ applied to ordination of microbial communities within each
 775 habitat-depth subset. Significant p-values are denoted by * (p < 0.05), ** (p < 0.01), and ***
 776 (p < 0.001). OTUs assigned to the following phyla were excluded from the 18S rRNA dataset
 777 prior to analysis: Holozoa, Metazoa, Chloroplastida and ‘NA’. Phenol=concentration of
 778 phenolic compounds; P-Ox=phenol oxidase activity; %Moss=percentage cover of mosses;
 779 %Grasses=percentage cover of graminoids; %Shrubs=percentage cover of shrubs;
 780 CO₂=carbon dioxide flux; CH₄=methane flux.

781

Marker	Variable	Bog- 5cm		Bog- 20cm		Fen- 5cm		Fen-20cm	
		R ²	p	R ²	p	R ²	p	R ²	p
16S rRNA gene	Core	0.71	0.001**	0.27	0.002**	0.07	0.2	0.14	0.04*
	pH	0.02	0.6	0.03	0.5	0.36	0.001**	0.12	0.045
	Phenol	<0.01	0.9	0.03	0.5	0.22	0.005**	0.35	0.001**
	P-Ox	0.14	0.024*	0.17	0.01*	0.02	0.6	0.05	0.3
	%Moss	0.23	0.002**	0.41	0.001**	0.08	0.1	0.23	0.002**
	%Grasses	0.17	0.017*	0.30	0.001**	0.20	0.006**	0.50	0.001**
	%Shrubs	0.59	0.001**	0.11	0.052	NA	NA	NA	NA
	CO₂	0.12	0.048*	0.11	0.058	0.02	0.6	0.15	0.02*
	CH₄	0.04	0.3	0.0174	0.6	0.04	0.4	0.07	0.2
18S rRNA gene	Core	0.04	0.4	<0.01	0.8	0.05	0.3	0.17	0.008**
	pH	<0.01	1	<0.01	0.9	0.01	0.7	0.27	0.001**
	Phenol	0.11	0.07	0.2	0.005**	0.10	0.06	0.20	0.006**
	P-Ox	0.05	0.3	0.04	0.4	0.05	0.3	0.19	0.009**
	%Moss	<0.01	0.8	0.01	0.7	0.08	0.1	0.27	0.001
	%Grasses	0.02	0.7	0.05	0.3	<0.01	0.9	0.03	0.5
	%Shrubs	0.05	0.3	0.08	0.1	NA	NA	NA	NA
	CO₂	0.03	0.4	0.01	0.8	0.04	0.4	0.08	0.10
	CH₄	<0.01	0.8	0.05	0.3	0.05	0.3	<0.01	0.9

782

783 **Table 2** Summary of the number and taxonomic affiliation of significantly drought-affected
 784 OTUs in sequencing datasets from each habitat and at each depth. Drought-affected OTUs
 785 shown were significantly affected by the treatment: time point interaction effect at a p-value
 786 of <0.05 prior to the application of corrections for multiple comparisons. Only taxonomic
 787 annotations with a utax confidence value of >0.85 are included, with annotations at lower
 788 confidence values classed as ‘unassigned’.

Marker	Habitat	Positive Effect	Negative Effect
16S rRNA gene	Bog-5cm	Proteobacteria (1); Acidobacteria (2); Bacteroidetes (1)	None
	Bog-20cm	Acidobacteria (2)	None
	Fen-5cm	Acidobacteria (1); Bacteroidetes (2); Proteobacteria (11); Unassigned Bacteria (3)	Pacearchaeota (1); Bacteroidetes (12); Firmicutes (1); Proteobacteria (4); Unassigned Bacteria (2)
	Fen-20cm	Acidobacteria (1); Unassigned Bacteria (1)	Unassigned Bacteria (3)
18S rRNA gene	Bog-5cm	Rhizaria (2); Unassigned Eukaryote (1)	None
	Bog-20cm	None	None
	Fen-5cm	Alveolata (1); Nematoda (1); Rhizaria (1); Unassigned Eukaryote (2)	Unassigned Eukaryote (2)
	Fen-20cm	Strameopiles (1)	None

789

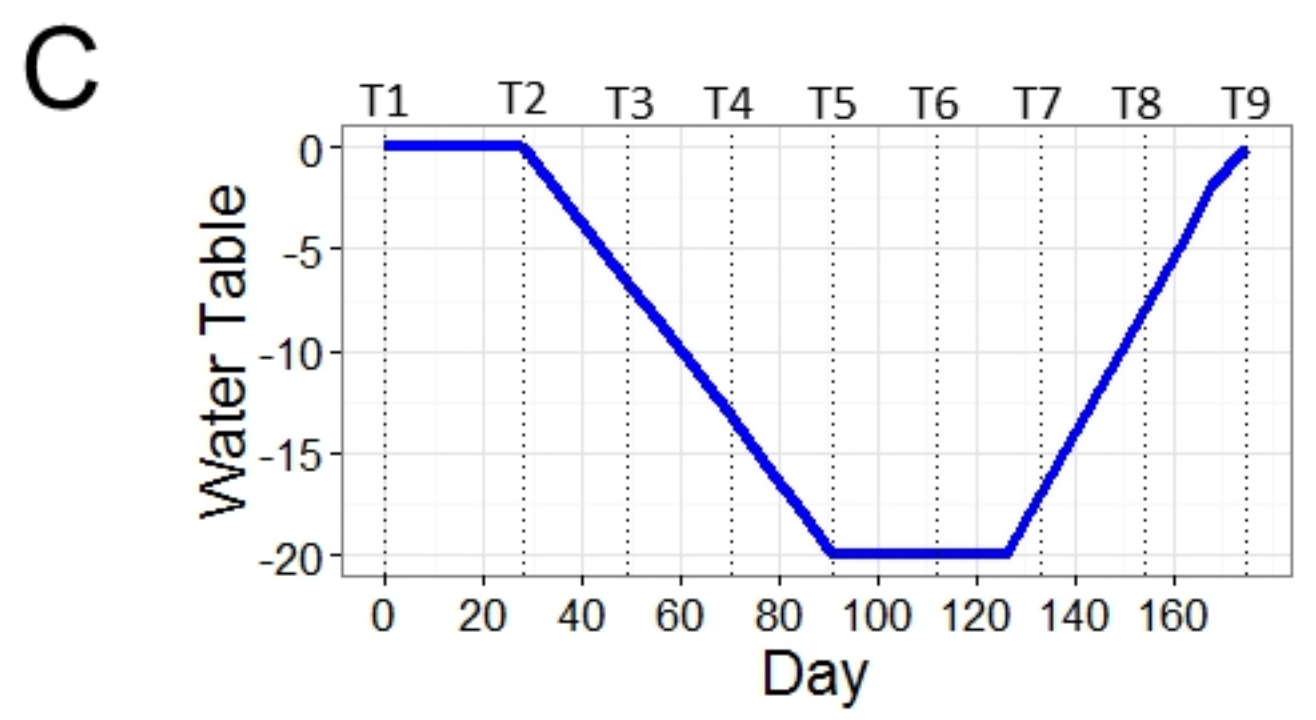
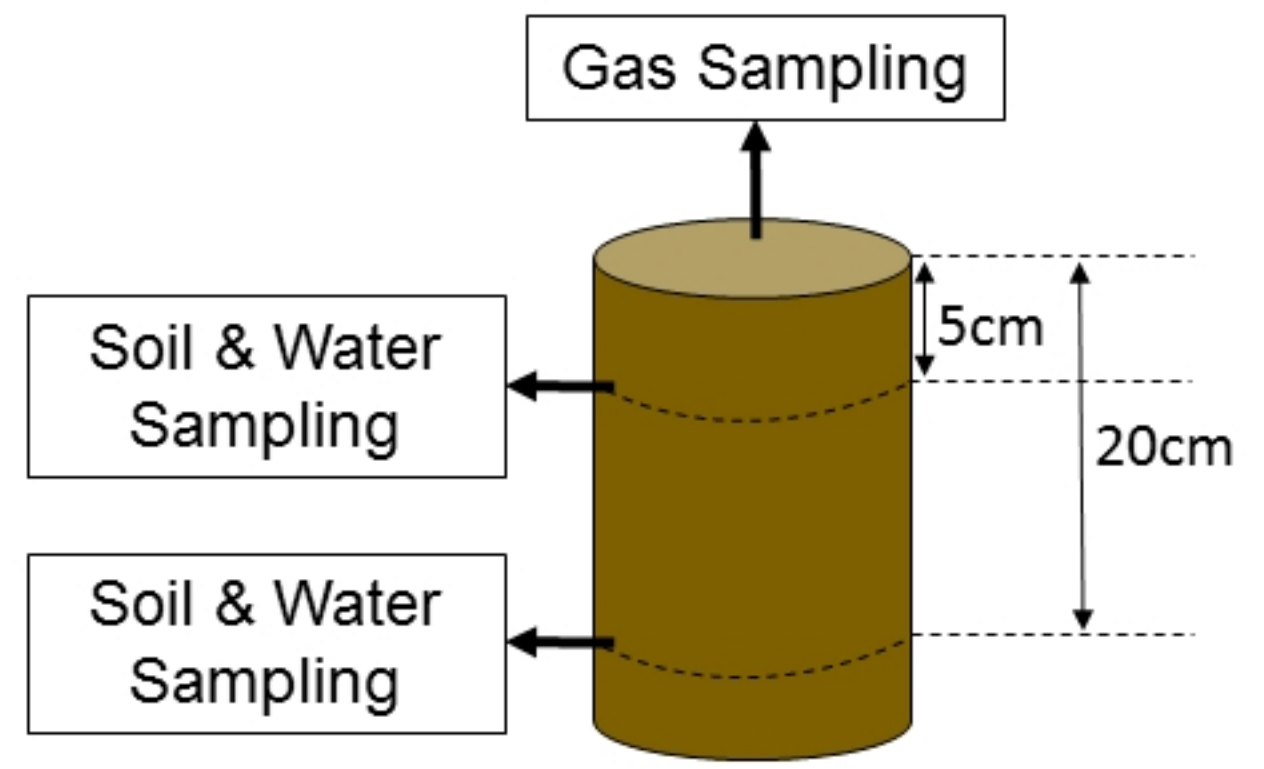
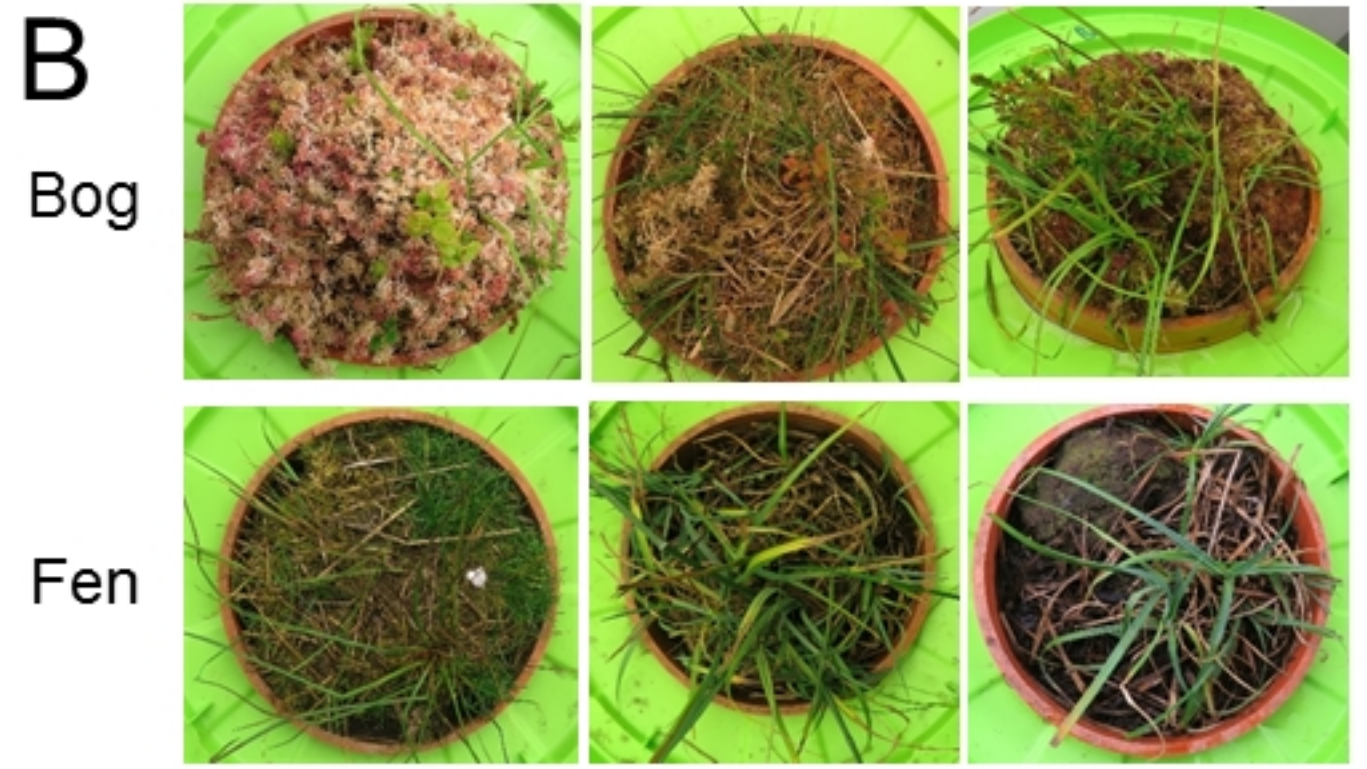
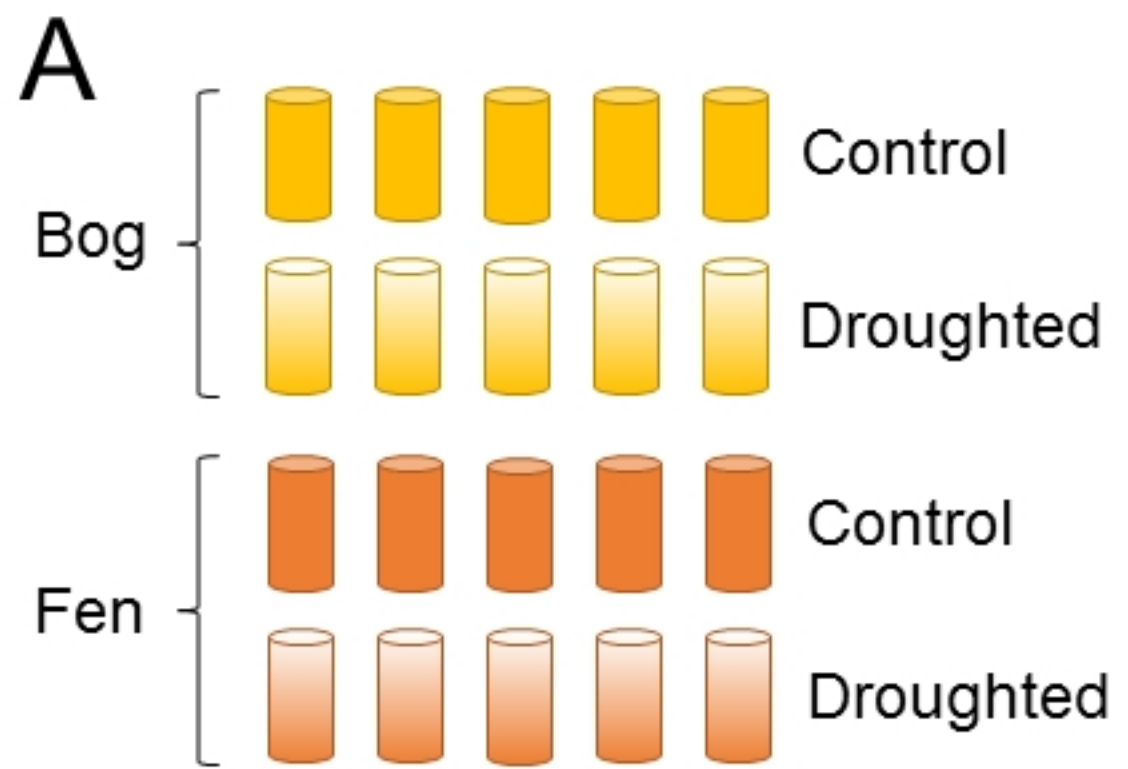
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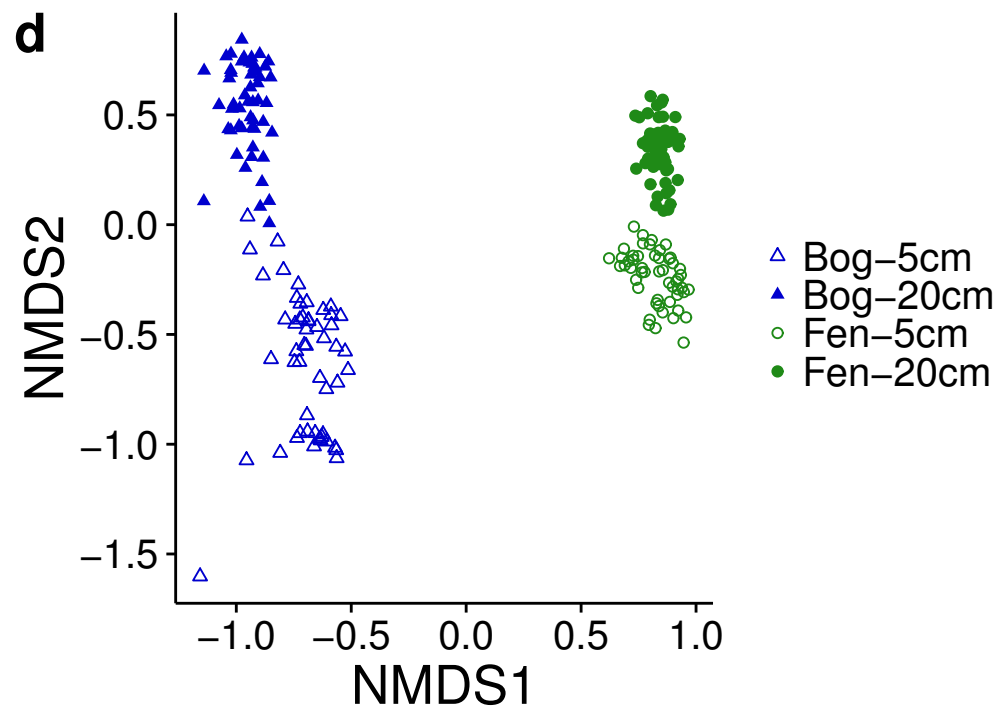
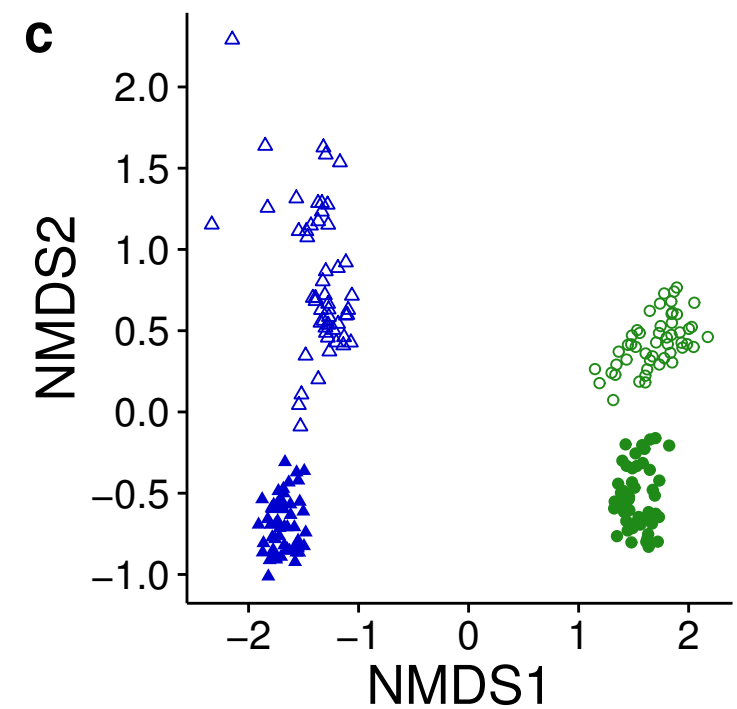
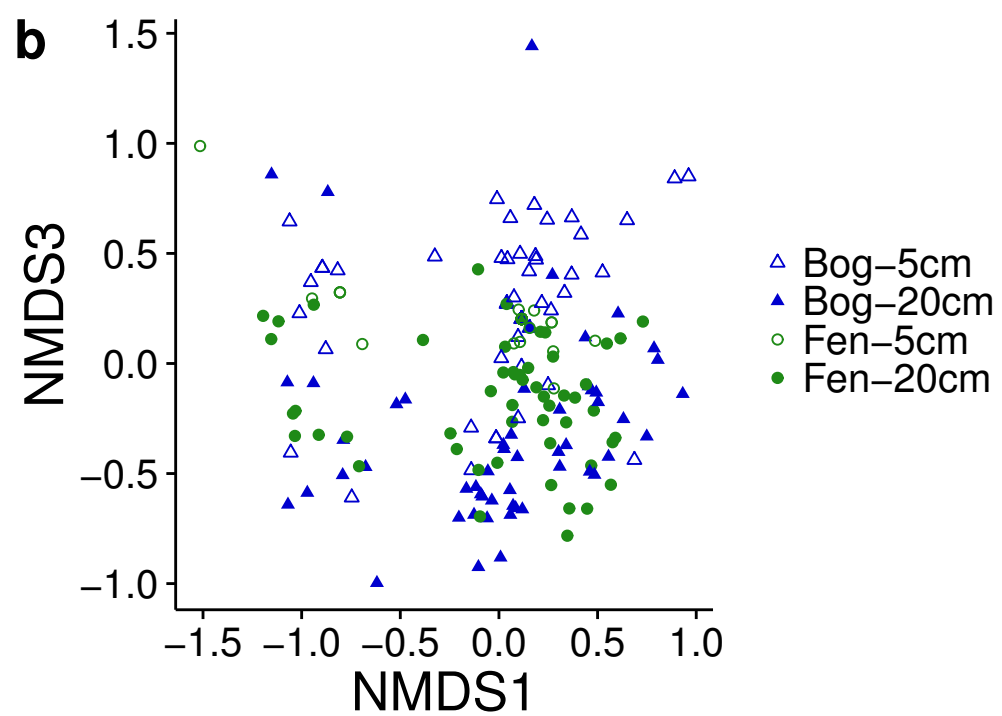
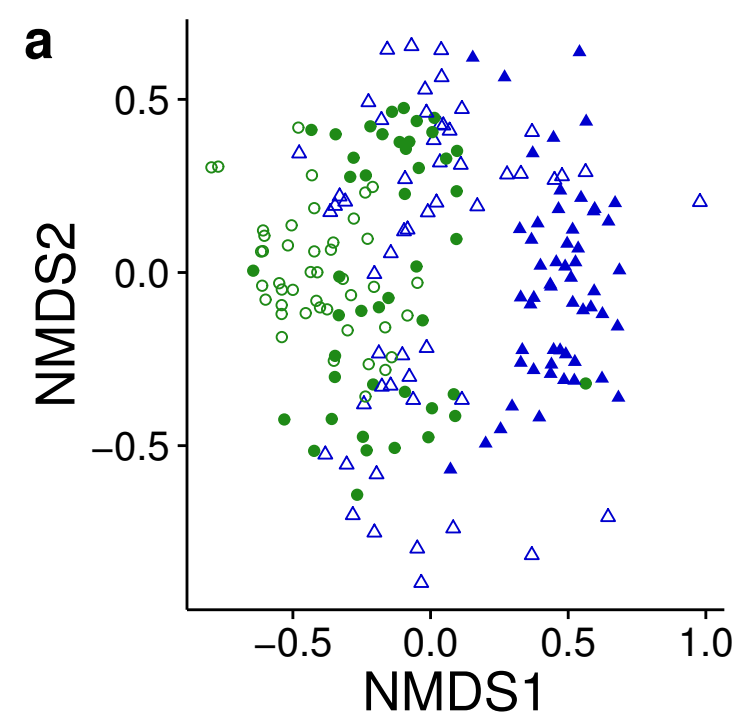
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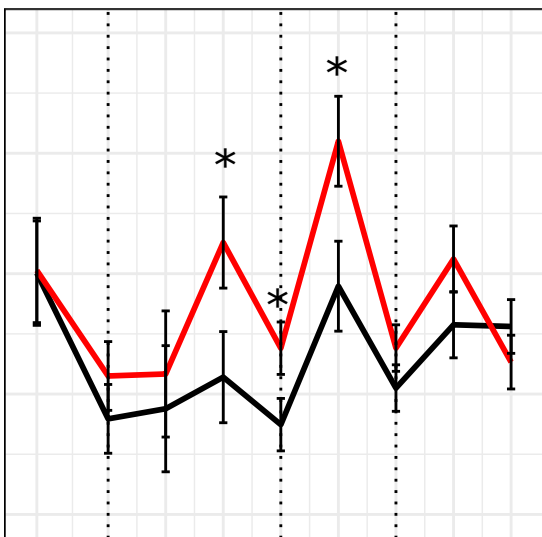
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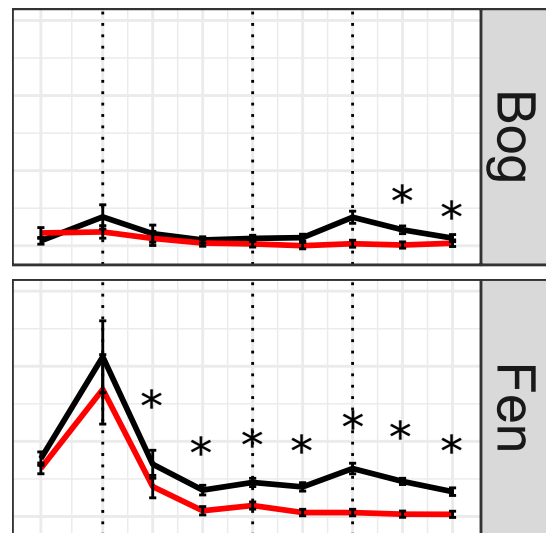




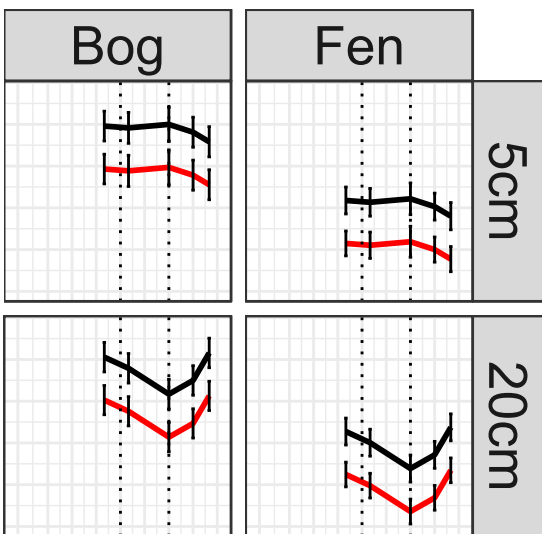
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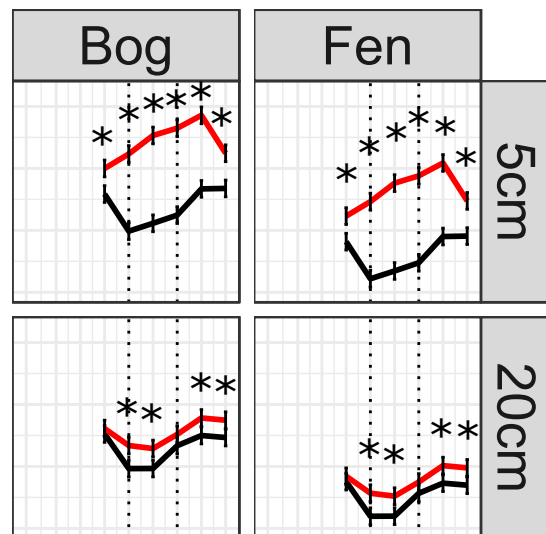
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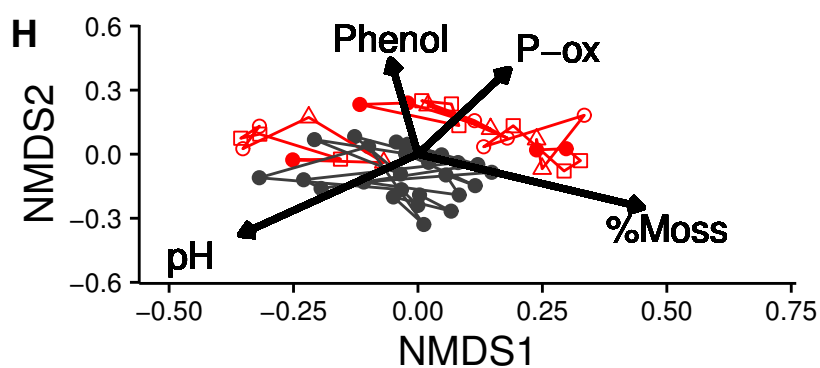
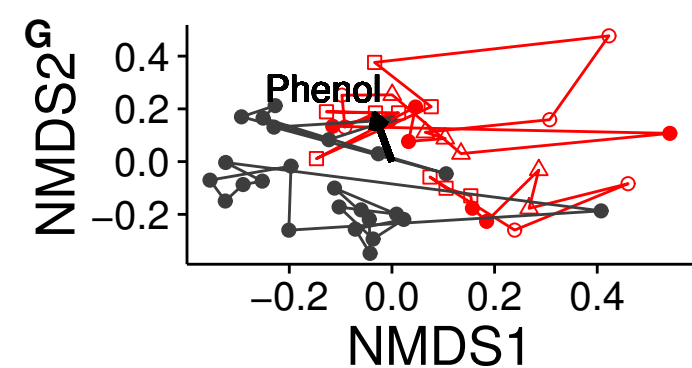
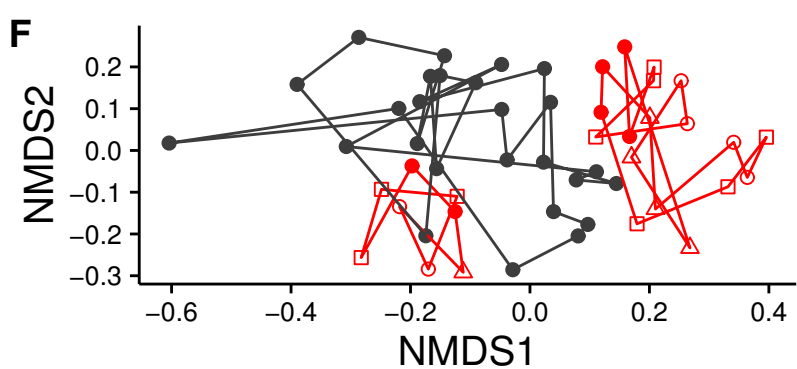
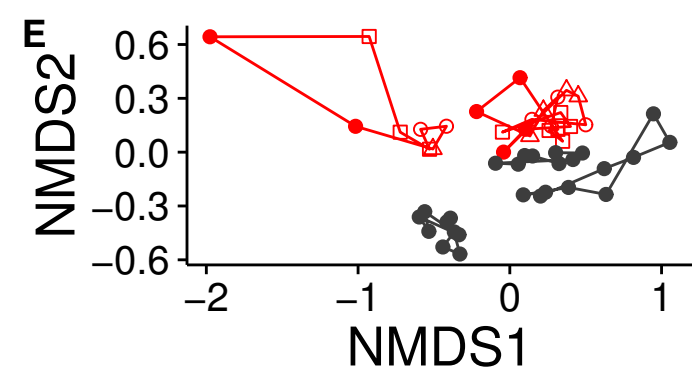
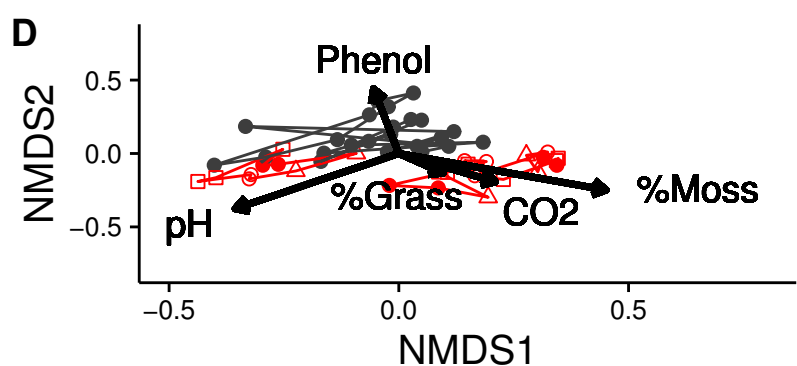
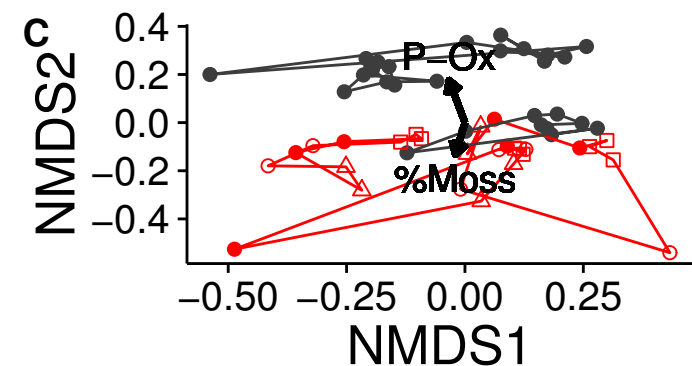
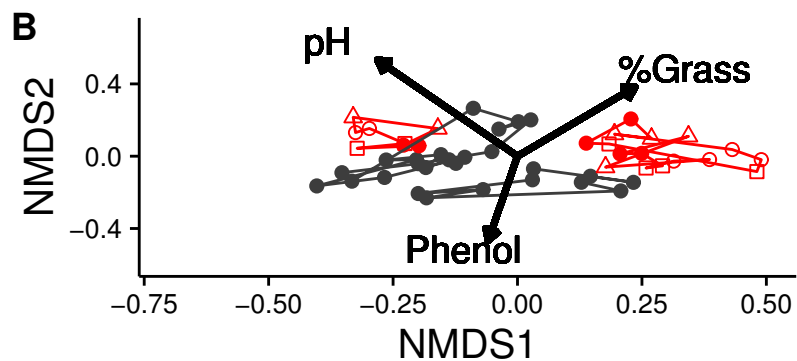
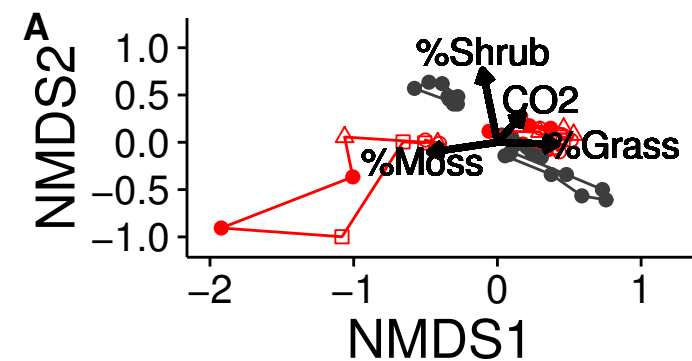


(c)



(d)





● Control
● Drought

WaterDirection

● Steady

□ Drying

○ Minimum

△ Rewet

Treatment

● Control

● Drought

WaterDirection

● Steady

□ Drying

○ Minimum

△ Rewet

Treatment

● Control

● Drought

WaterDirection

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